

**1409-Pos Board B360****Development of a Model for Excitability Studies using *Xenopus* Oocytes**

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Action potentials (AP) are basic functional units of electrical signaling in excitable cells. These electrical signals are involved in many biological processes, including muscle contraction, synaptic transmission and hormone release. In general, the plasma membrane is polarized, displaying a difference in electric potential (membrane potential) with a negative intracellular voltage with respect to the extracellular space. During an AP, the membrane potential is momentarily canceled (depolarized) or reverted (anti-polarized) by a inwardly-rectifying current typically mediated by sodium-selective voltage-gated channels (VGC); the membrane potential is returned back (repolarize) to its initial voltage (resting potential) by a outwardly-rectifying current mediated by potassium-selective VGC. The temporal and electrical characteristics of APs depend on which VGCs are present in the membrane. Understanding the role of VGCs in AP generation in their native cells constitutes a difficult task, commonly riddled with the use of pharmacological agents to isolate each specific conductance. Here, we have developed a model to study cellular excitability using *Xenopus* oocytes. Spontaneous and evoked APs were readily recorded from oocytes expressing Nav1.4, *Drosophila* Kv1.1 (Shaker), human Kv7.2 and Kv7.3. These APs were around 5-ms long. However, in the absence of Shaker, the AP lasted about 50 ms. These observations indicated that we were able to modify the temporal characteristic of APs by removing the fast-activating Shaker. To further validate this model, we used the Kv7.2-7.3 agonist diclofenac seeking to decrease excitability. The addition of diclofenac drove the resting potential to more negative voltages and raised the threshold for excitation, effectively decreasing excitability. These results constitute proof of concept showing that this type of models can be used as functional scaffolds for the evaluation of pharmacological agents and the assessment of the effect of mutations in VGCs on the generation of bioelectrical signals.

**1410-Pos Board B361****Localization of the *P. falciparum* K<sup>+</sup> Channels (PFKCH1 and 2) and Functional Expression in Yeast**

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The causative agent of malaria is the unicellular protozoan *Plasmodium*. The parasite has a complex life cycle, involving asexual replication in human red blood cells (RBCs) as well as sexual replication, forming egg-like cells called oocysts in the mosquito vector. Increasing resistance towards known antimalarial drugs poses a significant problem in the fight against malaria. Therefore, the development of novel drugs that target vital proteins encoded by the parasite has attracted major attention. *Plasmodium falciparum*, the species responsible for the majority of malaria-associated fatalities, encodes two putative K<sup>+</sup> channels, PfkCh1 and PfkCh2, which have been cloned in our laboratories. Although viable in all intraerythrocytic stages, Kch1-null *P. berghei* parasites exhibit a total inhibition of oocyst development in the mosquito midgut. Thus, Kch1 might serve as a potential target in novel parasite transmission-blocking strategies. In the present study, polyclonal antibodies were raised against the PfkCh1 and PfkCh2 channels. Our results demonstrate that Kch1 is located in the parasites plasma membrane in all blood stages of malaria, whereas Kch2 may be located to the parasite plasma membrane as well as to the RBC membrane. It has not been possible to express the *Plasmodium* K<sup>+</sup> channels in mammalian cells or *Xenopus* oocytes. Here we show that GFP-tagged PfkCh1 as well as PfkCh2 can be expressed in *Saccharomyces cerevisiae* at high levels. Complementation assays in *S. cerevisiae* revealed that the heterologously expressed channels could rescue the high potassium requirement of a *trk1Δtrk2Δ* yeast strain proving that the channels are expressed in a functional state.

**1411-Pos Board B362****Sevoflurane Potentiates Kv Channels by Inhibiting a Late Non-Conducting State: A Plausible Mechanism of General Anesthetic Action Implicating the Selectivity Filter**Shelly T. Jones<sup>1</sup>, Juliana Hosoume<sup>2</sup>, Leticia Stock<sup>2</sup>, Caio Souza<sup>2</sup>, Werner Treptow<sup>2</sup>, Manuel Covarrubias<sup>1</sup>.<sup>1</sup>Neuroscience, Sidney Kimmel Medical College of Thomas Jefferson University, Philadelphia, PA, USA, <sup>2</sup>Laboratório de Biologia Teórica e Computacional, Universidade de Brasília, Brasília, Brazil.

Previous work has shown that sevoflurane potentiates gating of Kv channels, including mammalian Kv1.2, Kv1.5 and *Drosophila* K-Shaw2 (J Biol Chem, 287: 40425-40432, 2012). Kinetic modeling additionally showed that this potentiation could result from the elimination of non-conducting states connected to the open state but outside the main activation pathway. To test this hypothesis, we examined the effect of sevoflurane on K-Shaw2 inactivation. Consistent with the presence of a classical P/C-type mechanism, K-Shaw2 inactivation increases Na<sup>+</sup> selectivity and is inhibited by elevated external K<sup>+</sup>. To ask whether sevoflurane inhibits K-Shaw2 P/C-type inactivation, we investigated the positive reversal potential shift that occurs as inactivation develops. As expected for an increase in relative Na<sup>+</sup> selectivity induced by P/C-type inactivation, initial results showed that the magnitude of the inward tail current increases as the outward current slowly decays, and that 1 mM sevoflurane does not affect this relationship (albeit the outward peak current at strongly depolarized voltages increases as shown previously). Thus, K-Shaw2 potentiation by sevoflurane does not result from inhibition of P/C-type inactivation. Alternatively, inhibition of a flicker non-conducting state outside the main activation pathway might explain the potentiation by sevoflurane. Preliminary unitary current recordings of the K-Shaw2 channel indicate the presence of a short-lived flicker state. We will present additional K-Shaw2 single channel results and Kv1.2 molecular dynamics simulations, which more directly implicate the selectivity filter region as a possible site of sevoflurane action. Supported by NIH Grant P01 GM055876-14.

**1412-Pos Board B363****Gβγ Subunits Modulation of Kv7.4 Channels Expressed in HEK293 Cells at the Single Channel Level**

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Recently it was shown that KCNQ4-encoded voltage-dependent potassium channels (Kv7.4) play an important role in the regulation of resting vascular tone and in response to endogenous vasodilators (*Hypertension*, 59:877-884, 2012). However there is lack of information about Kv7.4 basic biophysical properties on the single channel level and about their regulatory mechanisms. Therefore, using standard cell-attached and inside-out configurations of the patch-clamp technique we investigated the single channel properties of Kv7.4 in HEK293 cells stably expressing the channel. We found that NPo of Kv7.4 was  $0.61 \pm 0.05$  (n=9, mean  $\pm$  SEM) in the cell-attached configuration at -50 mV (voltage applied to the patch pipette), whereas in the inside-out configuration at 0 mV NPo of Kv7.4 was  $0.04 \pm 0.01$  (n=9). Based on the inside-out experiments, single channel conductance of Kv7.4 was calculated as 2.31 pS. Linopirdine (10  $\mu$ M), the pan-Kv7 blocker, abolished the single channel activity in both cell-attached and inside-out configuration when added to the patch pipette, but did not produce significant changes when applied to the bath solution. Bath application of PI(4,5)P<sub>2</sub> (1  $\mu$ M) to the inside-out patches or application of active Gβγ subunits (50 ng/ml) resulted in significant increase of NPo of Kv7.4 correspondingly to  $0.27 \pm 0.09$  (n=3) or to  $0.34 \pm 0.06$  (n=6) without affecting channel conductance (p<0.001 in both cases, determined by 2-way ANOVA). Moreover, bath application of gallein (100  $\mu$ M), an inhibitor of Gβγ activity, reduced significantly NPo of Kv7.4 channels to  $0.03 \pm 0.01$  (n=3, p<0.001) in the cell-attached experiments. Apart from revealing the basic biophysical properties of single Kv7.4 channels our results show that, similar to PI(4,5)P<sub>2</sub>, Gβγ subunits play a critical role in the basal activity of voltage-dependent Kv7.4 channels.

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